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TECHNOLOGISTS OF SOUTH AFRICA

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EDITORIAL

The South African Journal of Medical Laboratory Technology is the organ through which medical laboratory technologists here demonstrate the original work they are undertaking and have produced.

The opportunity in South Africa for original research in medical laboratory technology is wide. There is a wealth of medical research being published in medical journals throughout the world, some of which work is being produced by South African medical officers and scientists.

It is time that the medical laboratory technologist here took stock of his position. He is desirous of higher academic status, a status equivalent to a B.Sc. degree. To agitate for such status is one thing. To prove oneself worthy of it is quite another.

A Journal such as ours should have a waiting list of contributors—it should not be necessary to solicit members to write papers in order to keep the Journal going. To have to do so means that there is lacking among technologists here either the initiative or ability to do original work.

It is certainly not the prerogative of the senior technologist to produce papers; any member or technologist with some experience can undertake a paper, and these people would have the fullest co-operation and help from both their seniors and the heads of department.

Copies of the Journal are sent all over the world; let us make it an organ which is eagerly awaited, an organ which offers a definite contribution to the science of Medical Laboratory Technology.

REDAKSIONEEL

Die Suid Afrikaanse Joernaal vir Mediese Laboratorium Tegnologie is die orgaan waarin mediese tegnoloë oorspronklike werk behoort te publiseer.

In Suid Afrika is velerlei geleenthede vir oorspronklike ondersoek in mediese laboratorium tegniek. 'n Ontsaglike volume van ondersoek word jaarliks in die wêreld gepubliseer en 'n gedeelte van die publikasies is afkomstig van Suid Afrikaanse mediese personeel en wetenskaplikes.

Die tyd het aangebreek vir die plaaslike mediese tegnoloog om sy posisie in heroorweging te neem. Hy is begerig om sy akademiese status te verhoog; dieselfde status as 'n B.Sc. graad in die natuurwetenskappe. Om vir die status te straf is 'n doel; om te bewys dat hy so 'n status waardig is, is 'n ander kwessie.

'n Joernaal soos hierdie behoort 'n waglys van aspirant publiseerdes te hê. Dit behoort nie nodig te wees om met die hoed in die hand rond te gaan vir oorspronklike publikasies nie. Die feit dat dit gedoen moet word skep die indruk dat tegnoloë die nodige drang na oorspronklike werk kwyt is, alternatief, dat die vermoë daartoe in gebreke bly.

Dit is geensins die voorreg van die senior tegnoloog om oorspronlike werk te onderneem en publiseer nie. Enige tegnoloog met ondervinding kan oorspronklike werk aanpak en sal op die hulp van die seniors en departementele hoofde kan staatmaak.

Hierdie Joernaal word wêreldwyd versprei. Laat ons dus 'n poging aanwend om dit 'n orgaan te maak wat verwelkom word en 'n daadwerklike bydrae tot mediese laboratorium tegnologie lewer.

A COMPILATION OF RICKETTSIAL DISEASES IN SOUTH AFRICA AND THEIR SEROLOGICAL LABORATORY DIAGNOSIS

By V. G. ALBERTO
(Union Health Dept., Durban)

Epidemic typhus has been known for centuries but it was only in 1909 that Nicolle and his colleagues (1911) first discovered that infection was spread by the louse Pediculus humanus. It was not until 1916 that da Rocha-Lima demonstrated the aetiological agent of the disease and named it Rickettsia prowazeki in honour of Ricketts and Prowazek, both of whom succumbed while studying the disease.

Although Ricketts in 1907 had succeeded in transmitting Rocky Mountain spotted fever to guinea pigs, the generic relationship of this disease to typhus fever remained obscure till Wolbach in 1916 demonstrated the rickettsial nature of the causative agent.

During the war of 1914-1918, a new fever referred to as Trench fever was met with; this was likewise found to be due to a species of Rickettsia. A sporadic form of typhus fever had been recognised for a long time in New York, a severer form known as Tabardillo in Mexico and a milder form in Manchuria and the Far East. Broadly speaking, until 1925, typhus fever was regarded as one of the major epidemic diseases, which in certain areas might sometimes occur as a relatively mild sporadic form. Rocky Mountain spotted fever and Trench fever were regarded as rickettsial diseases due to a species of Rickettsia entirely different from that causing typhus fever.

In 1925 Fletcher and Lesslar found that the endemic typhus fever of the Federated Malay States could be divided serologically and epidemiologically into two entirely different groups, one met with in towns and the other in country districts. Both differ from the classical typhus and from each other.

The result has been to reveal the existence in several different countries of endemic typhus fever or typhus-like fevers many of which had previously been unrecognised.

These fevers are broadly divided into four groups on the basis of clinical features, epidemiological aspects, and serological and immunological characteristics :—

1. The typhus fever group.
2. The spotted fever group.
3. The Japanese river fever or Tsutsugamushi group.
4. Q-fever to which will be added Trench fever.

In Southern Africa four rickettsial diseases are known to affect man. These are :—

1. (a) Epidemic louse-borne typhus fever. (Typhus Fever Group.)
   (b) Murine flea-borne fever. (Typhus Fever Group.)
2. Tick-bite fever. (Spotted Fever Group.)
3. Q-fever.

There is serological evidence of the occurrence of Rickettsial pox in Southern Africa but this evidence still has to be strengthened by the isolation of the causative rickettsiae from patients and from the vector or vectors.

General Characteristics of Rickettsiae

The rickettsiae differ from most bacteria in being able to grow only within living cells and in this respect they are more closely related to the filtrable viruses. However, they differ from these filtrable viruses insofar as most of them are slightly too large to pass through fine bacteriological filters. The virus of Q-fever is the only pathogenic one known to be definitely filtrable.

Morphologically the organisms are diplococcoid, vary in size from almost invisible particles to 2u in length. Stained with Giemsa they take a purplish tint and with Castenada's stain they are light blue in colour. They are Gram negative, not cultivable on ordinary media but grow in the cells of tissue cultures. All the known pathogenic species occur intracellularly with the exception of Trench fever.

It must be acknowledged that Trench fever has not yet been demonstrated by scientific experiment and also that lice fed on healthy people have developed extracellular rickettsiae apparently identical to those observed in Trench fever.

Brief History of the Weil-Felix Reaction

Since the demonstration of the causative organism by growth in tissue culture, the chick embryo of the louse's gut is a lengthy procedure, possible only under favourable conditions, recourse is had mainly to the agglutination test on the patient's serum known as the Weil-Felix reaction.

In 1916 Weil and Felix, working in Eastern Galicia, cultivated from the urine of a patient with typhus, a Proteus-like organism that had the peculiarity of being agglutinated not only by the patient's serum but also by the sera of other patients suffering from typhus fever. Similar organisms were isolated from the urine and blood of other patients suffering from typhus and these organisms were called Proteus X strains; the most highly agglutinable and most specific for typhus being the Proteus OX19 strain (Felix 1916). This strain was agglu-

tinated to a titre varying from 1/50 to 1/50,000 by typhus sera but never above 1/25 by sera from non-typhus patients.

The important antigen for the diagnosis of Japanese river fever is the Proteus OXK strain. Fletcher and Lesslar (1925, 1926) studying the endemic typhus of the Federated Malay States, observed that OX19 was positive in some cases but not in others. Wondering whether their strain of OX19 was losing its agglutinabality, they tried a fresh strain which had been brought out to the Straits Settlements by Dr. Kingsbury and which was called Proteus OXK. They found that this strain agglutinated with the sera of patients which failed to react to OX19. It was further observed that the sera which reacted with OX19 did not agglutinate OXK. Further work showed that the typhus patients whose blood agglutinated OX19 were to be found mainly in urban areas while those whose blood agglutinated OXK came from the country districts, especially where rank grass and scrub had grown up. Fletcher referred to the former as urban or shop typhus and to the latter as rural or scrub typhus. The shop typhus is of the ordinary endemic murine type whereas the scrub typhus appears identical to the Tsutsugamushi disease of Japan. Experience has confirmed the value of OXK in the diagnosis of Japanese river fever. Proteus OXK, however, tends to be agglutinated more often and to a higher titre by normal sera than OX19, so that complete agglutination in a final dilution of 1/200 should be demanded for diagnostic purposes. Even then great care must be exercised in interpreting the result.

A strain of Proteus X corresponding to the virus of the Spotted fever group has not yet been discovered, but a reaction often not appearing until late in the second week, may occur with one or more of the Proteus OX19, OX2 or OXK strains. Between the 10th and 32nd days of illness, 77% of sera agglutinated OX19 to 1/160 or over. A relatively high OX19 and a relatively low OX2 titre is the most frequent combination found. Such a result or OX19 alone, may be due to typhus fever. On the other hand, a high OX2 titre either alone or with a low OX19 titre is suggestive of spotted fever. OXK is usually agglutinated least of all.

Rickettsial Diseases in South Africa

Firstly, we will consider rickettsial diseases occurring in Southern Africa and then we will briefly consider the others for the sake of completeness.

Epidemic Typhus Fever

Synonyms : Jail fever; ship fever; putrid fever; petechial fever; typhus exanthematicus; Ger. Fleckfieber; Fr. typhus exanthematique; Sp. El tabardillo; Ital: typho-esantematico.

Causative agent : Rickettsia prowazeki.

Vector: Pediculus humanus (Louse).

Introduction: Typhus fever is an acute infectious disease with an incubation period of 10 to 14 days. There is a fairly abrupt onset, with a continued fever lasting 2 weeks, followed by a critical fall, or rather rapid lysis of temperature. About the fifth day a rose-spot eruption first appears about the loins and abdomen, later on extending over the trunk and extremities. The rash tends to become petechial and stands out rather prominently on a general cutaneous mottling. A stuporose state is a marked feature of the disease. It generally has a seasonal incidence, occurring during winter and spring. The mortality varies with age, viz.: the older the patient, the severer the disease. The case mortality in epidemics is about 20%.

Weil-Felix reaction: Agglutinins appear in the blood about the 4th day and increase to an acme about the end of the 2nd or beginning of the 3rd week. During convalescence they fall off rapidly and after 5 months have disappeared almost completely. 7 - 9% of normal persons react at a titre of 1/25 but generally an agglutination of 1/50 can be obtained by the 8th day for a positive diagnosis. As with most serum tests used in diagnosis, there are certain anomalies and limitations. Patients suffering from Proteus infections such as pyelitis, cystitis, cholecystitis, pleuritis and bronchitis as well as patients suffering from Proteus infected wounds have had titres of 1/200 to 1/1,600 against OX19. Fluctuating titres have been noted in patients suffering from undulant fever, enteritis, typhoid fever and dysentery. It follows that the result of the Weil-Felix test should be interpreted strictly in relation to the clinical findings. Generally speaking, except in endemic areas or in patients who have previously suffered from typhus fever or have been inoculated, complete agglutination of OX19 in a titre of 1/100 or over during the 2nd week of the disease is highly suggestive of active infection. Cases occur in which the antibody response is poor or transient and here the result is regarded as doubtful or negative. Repeated examinations at intervals of 3 - 4 days are of value in detecting transitory reactions and changes of titre are indicative of active infection. A strong reaction is regarded as a good prognostic sign; a positive reaction becoming negative during the course of the disease is regarded as indicative of severe toxaemia and a fatal outcome.

Diagnosis in inoculated patients does not differ materially from uninoculated persons. Chick embryo vaccine does not stimulate agglutinins for OX19. Residual agglutinins from other types of vaccines are usually present but in very low titres. A rising titre for OX19 therefore, is highly suggestive of active infection.

It is important to realise that agglutination of the O antigen alone of Proteus is indicative of typhus infection. Normal agglutinins to the H antigen of Proteus are not uncommon and have no significance in the diagnosis of typhus fever.

Although Proteus X organisms have been isolated from several typhus cases, they are neither causative organisms nor secondary invaders. The reaction is therefore heterologous and not specific.

Rickettsial complement fixation : Specific rickettsial agglutination and complement fixation tests have been introduced for their more specific and differential value. These tests usually, but not invariably, differentiate between louse typhus and flea typhus and so far have always distinguished tick-bite fever from either louse typhus or flea typhus. One disadvantage of these rickettsial tests is that the antibodies may be detectable for many years after the patient's illness, and so they do not differentiate recent from remote infection. Rickettsial antigens for complement-fixation tests are usually prepared from infected yolk sac tissue. It is therefore advisable to test all sera with normal yolk sac antigen. This will exclude nonspecific antibodies acquired from vaccination. However, as in all serological tests, the demonstration of a change from a negative to a positive result is of diagnostic value.

Serological results in a case of epidemic louse-borne typhus fever.

Agglutination of Porteus	OX19	1:3,200
	OX2	1:400
	OXK	1:100
Complement-Fixation with		
R. prowazeki (Epidemic typhus)		1:3,200
R. mooseri (Murine typhus)		1:800
R. conori var. pijperi (tick-bite fever)		0
R. akari (rickettsial pox)		0
R. burneti (Q-fever)		0

Serological results in a case of recrudescent epidemic typhus fever.

Agglutination of Proteus	OX19	1:50
	OX2	0
	OXK	1:50
Complement-Fixation with		
R. prowazeki (Epidemic typhus)		1:3,200
R. mooseri (Murine typhus)		1:800
R. conori var. pijperi (tick-bite fever)		0
R. akari (rickettsial pox)		0
R. burneti (Q-fever)		0

Note : It has been proved that Zinsser's concept of Brill's disease

as a recrudescence of an infection acquired many years earlier is correct. Several cases have been recognised in this country. They conformed to the classical picture occurring in individuals who were born in Eastern Europe, where louse-borne typhus fever occurred in epidemic form.

Endemic Typhus Fever.

Synonyms: Murine typhus.
Causative agent: Rickettsia mooseri.
Vector: Xenopsylla cheopis and X. astia. (rat fleas).

> **Note:** Zinsser and several other investigators have shown that murine typhus can also be transmitted from man to man by the louse, as well as the tropical rat flea.

Introduction: Except that the headache and the delirium are not so marked, and the rash tends to be evanescent, the clinical picture resembles that of epidemic typhus. However, endemic typhus fever generally has a seasonal incidence, occurring mainly during summer and autumn, and also a mortality for all ages of about 2%.

Weil-Felix reaction: See epidemic typhus fever.

Rickettsial Complement-Fixation:

Serological results in a case of murine flea-borne typhus fever.

Agglutination of Porteus	OX19	1:1.600
	OX2	1:100
	OXK	1:50
Complement-Fixation with		
R. prowazeki (epidemic typhus)		1:400
R. mooseri (murine typhus)		1:1,600
R. akari (rickettsial pox)		0
R. conori var. pijperi (tick-bite fever)		0
R. burneti (Q-fever)		0

Tick-Bite Fever (South African)

Causative agent: Rickettsia conori var. pijperi.

Vectors: Amblyomma hebraeum, Rhipicephalus appendiculatus, R. evertsi, Hyalomma aegypticum and all stages of the common dog tick Haemaphysalis leachi. Boophulus decolatus is said to be a vector although the causative organism has not been isolated from it in this country.

Introduction: A variety of tick typhus occurring in Southern Africa and characterised by a primary sore often, having a blackish necrotic centre, by regional lymphadenitis, and in most cases by

intermittent or remittent fever lasting 10 - 14 days and a maculopapular rash which appears on the 3rd to 5th day of illness and when profuse, typically involves the palms of the hands and the soles of the feet. Fatalities practically never occur.

Weil-Felix reaction: Positive serological results are rarely obtained before the 10th day of illness and the maximum titre is usually attained 2 - 4 weeks after defervescence. On the average OX19 and OX2 are agglutinated to approximately equal titres. In some cases, OX19 may be agglutinated to a higher titre than OX2. On these serological findings it is not possible to differentiate from louse and flea typhus. Cases in which OX2 is agglutinated to a higher titre than OX19 are almost certainly cases of tick-bite fever. For differentiation the complement-fixation test is used.

Rickettsial Complement-Fixation:

Serological results in a case of tick-bite fever.

Agglutination of Proteus	OX19	1:100
	OX2	1:800
	OXK	1:100
Complement-Fixation with		
R. prowazeki (epidemic typhus)		0
R. mooseri (murine typhus)		0
R. conori var. pijperi (tick-bite fever)		1:400
R. akari (rickettsial pox)		1:100
R. burneti (Q-fever)		0

Q-Fever.

Synonyms: Nine mile fever.

Causative agent: Rickettsia burneti.

Vector: Although R. burneti is a parasite of various species of ticks the infection is apparently not often transmitted by tick-bite. Most patients contract the disease in rural areas by association with cattle, presumably by inhalation of infected dust or by drinking infected milk.

Introduction: The incubation period varies from 2 - 4 weeks. The onset of the illness is usually sudden, occasionally insidious, with malaise, chilly feelings, headache and sometimes nausea and vomiting. The fever lasts from 1 day to several weeks, most often about 4 - 7 days. There is usually an interstitial pneumonitis.

Weil-Felix reaction: This reaction is negative.

Rickettsial Complement-Fixation: This usually does not become posi-

tive until the 2nd week of illness and the maximum titre is attained about 3 weeks after onset. Titres of 1/50 or over are of diagnostic value, but it is more convincing to compare the results of a specimen collected early in the illness with one collected later or in convalescence to demonstrate the appearance of antibodies or an increase in titre in the 2nd specimen.

Serological results in a case of Q-fever.

Agglutination of Proteus	OX19	0
	OX2	0
	OXK	0
Complement-Fixation with		
R. prowazeki (epidemic typhus)		0
R. mooseri (murine typhus)		0
R. conori var. pijperi (tick-bite fever)		0
R. burneti (Q-fever)		1:800

Rickettsial Pox.

Causative agent : Rickettsia akari.

Vector : Appears to be the mite Allodermanyssus sanguineus.

Introduction : A firm red papule appears at the site of the mite bite. This develops into a vesicle that shrinks and dries to form a black eschar. The regional lymph glands become enlarged and tender and about a week after the first appearance of the bite mark general symptoms of illness begin rather abruptly with chills, fever and sweats, backache and headache. The rash appears on the 3rd to 5th day of illness. At first it is maculopapular and then vesicles develop on the summit of the papules.

They dry to form black crusts, which ultimately fall off, producing scars.

Weil-Felix reaction : Of no value.

Rickettsial Complement-Fixation :

Serological results in a case of suspected rickettsial pox.

Complement-Fixation with :

R. prowazeki (epidemic typhus)	0
R. mooseri (murine typhus)	0
R. conori var. pijperi (tick-bite fever)	1:50
R. akari (rickettsial pox)	1:200
R. burneti (Q-fever)	0

Note : There is serological evidence of the occurrence of rickettsial pox in this country but this still has to be strengthened by the isolation

of the causative organism from patients and the vector or vectors.

The remaining rickettsial fevers not normally occurring in this country will be dealt with very briefly.

Spotted Fever of the Rocky Mountains

Synonyms : Rocky Mountain fever; Black fever : Blue disease.

Causative agent : Rickettsia rickettsi.

Vector : Dermacentor andersoni and D. variablis.

Weil-Felix reaction : A high OX2 titre either alone or with a low OX19 titre is suggestive of spotted fever.

Note : Fievre boutonneuse is the name of a fever of the spotted fever group occurring in the Mediterranean countries and it closely resembles S.A. tick-bite fever but is transmitted by the dog tick, Rhipicephalus sanguineus. Similar diseases occur in Kenya, India and South America and are transmitted by the family of ticks Ixodidae.

Japanese River Fever, Mite Fever, Tsutsugamushi.

Synonyms : Flood fever; Japanese river fever; Kedani mite disease; Shimamushi.

Causative agents : Rickettsia orientalis, R. tsutsugamushi and R. niponica. There is evidence that all three are the same organism.

Vector : Trombicula akamushi (Kedani mite).

Weil-Felix reaction : There is usually a positive agglutination in a moderately high titre with the strain OXK. There is no reaction with OX19 or OX2 and this is important insofar as excluding true typhus infection. Unfortunately, in some of the milder forms of the disease occurring outside Japan, these reactions are less constant and hence of less value.

Trench Fever.

Synonyms : P.U.O.; Meuse fever; Volhynian fever; shin fever; quintin or five day fever.

Causative agent : Rickettsia quintana has never satisfactorily been demonstrated in man but certain observations strongly suggest that this organism is responsible for the disease.

Vector : Pediculus humanus.

Weil-Felix reaction : This reaction is negative. There is no specific laboratory method of diagnosis but laboratory investigations are often of assistance in differentiating this disease from other infections such as malaria, European relapsing fever, tick fever, seven day fever and Weil's disease without jaundice.

Acknowledgment.

I wish to thank Mr. G. C. Buckle for the help and advice he has given me in the preparation of this article.

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Edited by Thomas M. Rivers.

PRIZE IN PARASITOLOGY

The Director of the Amoebiasis Research Unit, C.S.I.R./N.P.A./Univ. of Natal announces :—

(1) Entries are invited from members of the Society for a prize to be awarded for an essay not exceeding 2,000 words on the subject :—

"The Common Round Worm."

(2) Both qualified and student technologists are eligible.

(3) All entries shall be the property of the S.A. Journal of Medical Laboratory Technology.

(4) The entries shall be judged by a panel of three including the donor.

(5) The adequacy of documentation and illustration shall be taken into account. Such illustrations need not be drawn by the author, but acknowledgment must be made.

(6) Entries shall be in the hands of the General Secretary by 31st December, 1958.

PARASITISM IN PERSPECTIVE

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It is surprisingly difficult to give a simple, clearcut definition of parasitism. There are species of almost every major group of animals—and several of the major divisions of plants as well—which have adopted the parasitic way of life, and the ways in which they have adapted themselves to this type of existence are varied and many. Furthermore, many parasites are almost certainly still in the process of evolving a better relationship with their hosts and this further complicates the picture of parasites as we see them.

In order to get a clear concept of what a parasite is, it is helpful to consider the various interrelationships that are found in Nature between various plants and animals. Starting at the lowest level, within a species, individuals may be solitary—each living by itself and only coming together in pairs for mating. They may even be like the spiders where the male takes his life in both hands when he approaches the female to mate, as he may well get eaten before he makes the purpose of his visit clear. From this extreme of anti-social behaviour, we find individuals of a species being permanently associated in pairs, family groups or the vast-communities like the ants and bees—where tens of thousands of individuals live together and divide up the labour involved in living in a huge, single colony.

As with individuals of a single species, the associations between individuals of different species vary through a continuous series of relationships. Thus, like an elephant and a mole, they may totally ignore one another; like a lion and grass, they may be related through a food chain, in this case just one link being involved, a buck; or the one may be a predator and the other the victim—a relationship well known to all and having many well-known examples.

Besides these better known associations, there are a small group of interrelationships in which there are varying degrees of dependence between the individuals of two different species. These are termed symbiotic. The loosest of these relationships are lumped together under the name commensalism which literally means " eating at the same table." Associations grouped under this heading vary from such loose relationships as that found between the tick birds and the cattle on whose ectoparasites they feed, through various intermediate stages to associations which involve quite complex adaptations such as are found in the pilot fish which feeds on scraps of food left from a shark's kill. This little fish not only has a sucker for temporarily attaching itself to the shark, but also has evolved the nervous co-ordination which enables it to follow the exact movements of the shark when it is swimming.

The closest interrelationships in this group are termed mutualism. In these, both partners derive benefit and in fact, as a rule, neither can survive without the other. In the plant known as a lichen, the fungus supplies the inorganic materials and the alga, with its chlorophyll, photosynthesises starch; in wood-eating termites, intestinal flagellates digest the cellulose for which the termites have no enzyme and make the breakdown products available to their hosts.

Related to these latter two groups is the phenomenon of parasitism with which we are concerned; and few, if any, organisms have no parasites at all. In this relationship, the one partner is termed the host and the other the parasite. The parasite is usually very considerably smaller than the host—even a 60-foot tapeworm is far smaller than its human host—and the parasite is the only one which derives benefit from the association. The host, in lesser or greater degree, is harmed by its parasite by the loss of food, damage to its tissues, toxic products released by the parasite or by one or more of a number of other methods. It is interesting to note that it is probable that the bacteria in the human intestine—and there are very many types and large numbers—include commensals, which feed on the ingested food but apparently do no harm, parasites which cause disease and mutualists, which may help synthesise certain vitamins which the host absorbs.

Parasitism is a way of life in the same sense as any of the other ways described earlier and the abhorrence which we reserve for parasitic organisms is not really deserved. Many parasites have been evolving with their hosts over long periods of time and the perfectly adapted parasite does not usually wish to kill its host, in fact the opposite is almost always the case as death of the host usually means the death of the parasite as well. This feature of parasitism has been well expressed by the ecologist, Charles Elton, who wrote that, " The difference between a carnivore and a parasite is simply the difference between living on capital and income, between the burglar and the blackmailer. The general result is the same although the methods employed are different." Going further, there is really no vast difference between the antelope which feeds on living, growing vegetables and the parasite which feeds on excess food in the intestinal canal of its host. There are exceptions (e.g., anthrax and trichina) where death of the host is necessary for transmission of the parasite but these are extremely rare.

Living as it does in the midst of plenty, the parasite has dispensed with many appendages and organs essential for a non-parasitic existence. Thus most parasites, at least in the parasitic stages of their lifecycle, have lost locomotory organs, most of their sense organs and jaws

for masticating food. Some, like the tapeworms, have even dispensed with an intestine and absorb the predigested food in which they live through the entire body wall. Because of this loss of certain organs, it has even been suggested that viruses, if indeed they are living things, are an endpoint of parasitic specialisation as they have dispensed with almost all of the usual processes of life except reproduction. Whether this be right or wrong, from the point of view of evolution, parasitism is a dead end.

But the adoption of a parasitic life has not only meant a simplification of bodily structure. Parasites have had to evolve many adaptations, both morphological and physiological. Those parasites which live in the intestine have developed the ability to resist the actions of the enzymes by which they are surrounded and which normally digest carbohydrates, fats and proteins, such as the body of the parasite is composed of. For this purpose, the parasitic tapeworms have a special cuticle, absent in their free-living relations. Those which live in the bloodstream or other tissues of the body of their hosts are able to resist the defences of the body which include phagocytic cells and antibodies. The flattened bodies of fleas and the claws of lice are well evolved for their purpose. The former enable fleas to move easily through the hairs; the latter enable lice to grip the body hairs. Further interesting adaptations may be seen in the tapeworms which not only have developed suckers and hooks as holdfasts against the turbulent currents caused by the peristaltic constructions of the intestines, but are able to respire without free oxygen by breaking down glycogen and releasing carbon dioxide and fatty acids. The ability to live anaerobically is very common among endo-parasitic animals.

The many ways which parasites have evolved to ensure the continuation of their species are also very remarkable examples of adaptations to parasitism. It is often very difficult for the parasite to reach a new host. Writing in the book " Life " the authors conclude that " Successful completion of a parasite's life-cycle is so improbable that only one in a million makes it." In most species, fantastic numbers of eggs are produced. For example, a female *Ascaris lumbricoides* (the common roundworm of man) lays about 200,000 eggs per day. In quite a number of parasites, one or more intermediate hosts is utilised to enable the offspring to successfully reach the final host—in some of these, there may be further multiplication of each larva in the intermediate host to further enhance the chances of survival. Hermaphroditism is common among parasites necessitating only one organism for the continuation of the species. An interesting example of a unique type of reproductive adaptation is seen in the fish, *Photocorynus*, which lives in the darkest depths of the sea. The males attach themselves to the females at a young age and become true parasites.

It can thus be seen that parasites should not really be regarded as degenerate organisms as many books suggest. They are species which have adapted themselves by the loss or development of organs, habits or physiological processes to a particular way of life, and that way of life is no better or worse than those which are more familiar to us. Before looking down on parasites, it is also as well to bear in mind that for the first nine months of our existence as individuals, we are parasitic, and the same applies to the early stages of many plants.

FURTHER USES FOR KIRSCHNERS LIQUID MEDIUM

J. H. MAYTHAM,
Department of Bacteriology, University of Cape Town.

In a previous communication it was shown that Kirschners Liquid Medium was as efficient as the more commonly used biological test in guinea pigs for the insolation of Mycobacterium tuberculosis from routine laboratory specimens. (Maytham 1955.) Since that time this medium has been used for a variety of procedures some of which are reported in this paper.

The Direct Antibiotic Sensitivity Test

This is a slide test for testing the sensitivity of Myco. tuberculosis to Streptomycin.

Sputum No. 3357. Direct microscopic examination of this specimen revealed an average of 18 tubercle bacilli per oil immersion field.

Seven smears were made of this sample of sputum using approximately similar amounts of the specimen for each slide and towards one end of the slide. The slides were then placed in a large sterile petri dish and allowed to dry overnight in a 37°C incubator.

Next day the preparations were decontaminated with 6% sulphuric acid for 20 minutes and then well washed with sterile distilled water to remove all traces of the acid. Six of the seven slides were stood upright in wide mouth screw capped bottles each containing 25 ml. Kirschners Medium including varying amounts of Streptomycin. The seventh slide was kept as a control. The six bottles were prepared as follows :—

Bottle No. 1.	Kirschners Medium.	
Bottle No. 2.	Kirschners Medium and Streptomycin	0.1 ugm/ml.
Bottle No. 3.	Kirschners Medium and Streptomycin	0.5 ugm/ml.
Bottle No. 4.	Kirschners Medium and Streptomycin	1.0 ugm/ml.
Bottle No. 5.	Kirschners Medium and Streptomycin	5.0 ugm/ml.
Bottle No. 6.	Kirschners Medium and Streptomycin	50.0 ugm/ml.

After 5 days incubation at 37°C the slides were removed from the medium and dried in the incubator in petri dishes for some hours. These slide preparations and the control smear were then stained by Ziehl Neelsen's method and examined.

The end point or sensitivity of the organism is the smallest amount of Streptomycin that inhibits growth. Obvious growth is the finding of filamentous cording acid and alcohol fast colonies under low or high dry power of the microscope. If these colonies are not readily apparent the films should be examined with the oil immersion lens for small colonies which are recognizable for their tendency to branch and string out. The finding of single bacteria or small groups comparable with those detectable in the control slide indicates that no growth has taken place.

The results of this examination were recorded as follows :—

Slide from Bottle No.	**Average Number of organisms / field.**
1	Cording growth
2	Cording growth
3	16
4	14
5	19
6	18
Sputum control slide	16

From this it will be seen that this strain of Myco. tuberculosis was sensitive to Streptomycin in concentrations of 1.0 ugm/ml.

The Indirect Antibiotic Sensitivity Test

This is a cultural test for testing the sensitivity of Myco. tuberculosis to Streptomycin.

Pleural Fluid No. 2273. The direct microscopic examination of Ziehl-Neelsen stained preparations and the biological guinea pig test were both negative for tubercle bacilli. The Kirschner culture, however, was positive and this was confirmed by finding typical lesions containing acid and alcohol fast bacilli in a guinea pig inoculated with an appropriate sample of the culture.

For this indirect test Kirschners Medium was dispensed in seven 1-oz. narrow neck McCartney bottles each containing 10 ml. of the

medium. Streptomycin was added to each of the first six bottles to give concentrations of 0.1; 0.5; 1.0; 5.0; 10.0 and 50.0 ugm/ml respectively. The seventh bottle received no antibiotic and was the control. Colonies from the culture to be tested were removed from the Kirschners Medium and ground up in a Ten Broeck glass tissue grinder and suspended in Kirschners Medium to make an appropriate and obviously turbid suspension. This was allowed to stand at room temperature for 15 to 30 minutes to allow the large particles to settle. The suspension was then standardized to No. 2 of the McFarland scale and then further diluted 1 in 10 using Kirschners Medium as diluent.

To each of the seven bottles 0.2 ml. of this diluted bacterial suspension was delivered and at the same time a similar series of bottles was inoculated with a similarly prepared suspension of the standard H37Rv strain of Myco. tuberculosis. Both sets of bottles were incubated at 38°C for 14 days at which time the following results were recorded :—

Bottle No.			*Growth of 2273*	*Growth of H37Rv.*
1.	0.1 ugm/ml. Streptomycin		++++	++++
2.	0.5 ugm/ml.	,,	++	—
3.	1.0 ugm/ml.	,,	—	—
4.	5.0 ugm/ml.	,,	—	—
5.	10.0 ugm/ml.	,,	—	—
6.	50.0 ugm/ml.	,,	—	—
7.	Control		++++	++++

The sensitivity of the test organism to Streptomycin is 1.0 ugm/ml. and only very slightly less than that of the standard strain of Myco. tuberculosis.

Testing the Efficiency of Sputum Homogenisation, and Concentration Methods.

Sputum Nos. 5571, 5619 and 6232.

These specimens of sputum showed the presence of acid and alcohol fast bacilli morphologically identical with Myco. tuberculosis on examination of Ziehl Neelsen stained smears. In specimen Nos. 5571 and 5619 they were present in fair numbers but in specimen 6232 they were very scanty. The treatment of each specimen was the same. Each was well mixed, then divided into two parts, care being taken that there was an equal division of purulent and mucoid elements so that the parts should be as nearly comparable as possible.

To one part of each specimen was mixed an equal amount of 4% NAOH with brom-thymol blue as an indicator. To the second part of Specimens. Nos. 5571 and 5619 an equal amount of 6% H_2SO_4 was

mixed. To the second part of Specimen No. 6232 an equal amount of a mixture containing 1% sodium hydroxide and 1% sodium sulphite in distilled water with brom-thymol blue as an indicator was added and mixed. The treated specimens were incubated at 38°C for one hour with frequent shaking and mixing during this period. Thereafter each sample was neutralised with 6% sulphuric acid or 4% sodium hydroxide, with brom-thymol blue as indicator, as required. This was followed by centrifuging at approximately 2500 r.p.m. for 30 minutes and the supernatant fluids discarded. To the deposits 0.5 ml. Kirschners Medium was added and well mixed. Ten-fold dilutions were then made of each of the deposits using a separate sterile pipette for each dilution. From each of these dilutions 0.1 ml. was delivered into 1 oz. narrow neck McCartney bottles each containing 10 ml. Kirschners Meduim.

The bottles were incubated at 38°C. Observations of the growth of colonies of tubercle bacilli were recorded in the following way :—

Sputum No.	*Reagent*	*Dilution*					
		Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
5571	H_2SO_4 6%	++++	+++	++	27	3	0
	NAOH 4%	++++	++++	+++	+	14	2
5619	H_2SO_4 6%	+++	++	75	14	1	0
	NAOH 4%	++++	+++	++	+	17	2
6232	NAOH 4%	+++	++	+	20	2	
	NAOH 1% NA_2SO_3 1%	Contaminated	+++	++	72	8	

+ = More than 100 colonies

It would appear that the treatment of sputum with 1% NAOH and 1% NA_2SO_3 is slightly superior to treatment with either 4% NAOH or 6% H_2SO_4. The former is the method employed in this laboratory for routine purposes.

Tuberculotatic Tests

A simple scanning test for the bacteriostatic effect of a particular substance was carried out in the following way :—

Eleven 1-oz. screw-cap McCartney bottles each containing 10 ml. Kirschners Medium and 0.2 ml. of a finely dispersed suspension of H37Rv strain of Myco. tuberculosis were set up and to each was added varying amounts of the substance to be tested.

In the following example synthetic vitamin K (Kavitin 4.8 mg., Abbott) was assayed for Bacteriostatic effect, using unit volumes from a "50 dropper." The bottles were incubated at 38°C.

Bottle No.	*Drops of Katvin*	*Amount of Growth Present*
1	10	No growth
2	9	No growth
3	8	No growth
4	7	1+
5	6	2+
6	5	5+
7	4	10+
8	3	10+
9	2	10+
10	1	10+
11 Control	0	10+

This result showed that the bacteriostatic effect of Kevitin ceased at the concentration present in Bottle No. 5.

Care should be taken to ensure that the preparation tested contains no preservative or chemical which might have a bactericidal action. In this case the Kavitin was made up in sodium bisulphite and when tested at the concentration stated on the bottle was found to have no inhibitory action.

Discussion

The fact that such a multiplicity of media and techniques have been and are still being devised for the isolation and culture of tubercle bacilli indicates that none is wholly satisfactory. What is needed is a medium that can be easily and rapidly prepared in any laboratory; that supports the growth of small numbers of tubercle bacilli; that will give rapid growth of tubercle bacilli from pathological material; that affords optimal growth conditions for all types of the tubercle bacilli; that the morphology of the colonies on the medium should allow reliable type determinations; that differentiates between tubercle bacilli and saprophytes; that gives poor growth conditions for contaminating bacteria; that would be suitable for drug sensitivity tests; that could be used for bactericidal and bacteriostatic studies and could be used for viable counts.

Kirschners Medium has been shown in a previous paper to be very suitable for routine isolations of Myco. tuberculosis from pathological materials (Maytham 1955) and in the present communication evidence is provided that it is also a very suitable medium for a variety of other observations dealing with this organism.

The chief advantages of the medium are that it can be easily and rapidly prepared; that it supports the growth of small numbers of

tubercle bacilli, that it gives rapid growth of tubercle bacilli from pathological material; that it supports the growth of both bovine and human varieties; that the colonies have a distinctive morphology and are thereby easily recognisable; that the number of colonies give a fairly reliable indication of the number of viable bacilli in the original material and this makes it possible to use the medium for a variety of useful procedures; that it is suitable for the maintenance of stock cultures.

Summary

Kirschners Liquid Medium is used in this laboratory for routine isolation of the tubercle bacilli from pathological specimens.

This paper indicates a variety of procedures for which this medium has proved to be equally suitable.

Acknowledgments

I wish to thank the Acting Head of the Department of Bacteriology for permission to publish this work, and the Technologists in charge of the Departmental Media Laboratory for the media supplied.

References

Maytham, J. H.. (1955) S.A.J. Med. Lab. Tech. 1, No. 3, 4.

LETTERS TO THE EDITOR

16 Kyalami,
Topham Road,
Pietermaritzburg.
3rd March.

Editor,
The South African Journal of Medical Laboratory Technology.

Dear Sir,

The December 1955 Editorial of the Journal compared the lot of the " on call " technologist in Britain with his less fortunate colleagues in South Africa.

In March of the following year the editor reported that the Director of the Provincial Medical and Health Services had agreed to approach the Provincial Executive Committee to discuss the matter of payment for " on call " duty. Retrospective payment was mentioned, and hope lightened the phone-bound vigils and noturnal journeys of of the three a.m. centrifuge, and if it was the jingle of cash that

brightened our bloodshot eyes, let's face it as such, and not as some vague enrichment of the soul.

We must recognise that there is an opening for one of those do-it-yourself books entitled " Medicine for Money," thinking carefully about the price of a surgical instrument with its counterpart in the tool shop, and remembering that if the doctor leaves his car lights on during a night call to a patient, the patient will pay for the new battery. A mercenary approach need not lessen the technologist's value to medicine. Shakespeare left a wealth of philosophy, but Will wrote for cash.

In going for the cash, it is my opinion that we may do better for ourselves if we co-operate with others in the same grind. Radiographers are similarly expected to extend their working hours without payment. A combined approach from the Medical Auxiliaries may yield better results, and the following points may be of value to the negotiating committee :—

1. Call duties restrict the free time of medical auxiliaries by approximately 40%.
2. The basis cost of a telephone is £8 a year.
3. It is not possible to compensate with time off and at the same time maintain a satisfactory daily service.
4. That one hour's work at 2 a.m. is the equivalent of a night's sleep, and therefore an occupational hazard.

Furthermore it might be asked of a patient (a reader of psychological articles in Reader's Digest) whether he would prefer to have his blood grouped by a contented or disgruntled technologist, whose skill and diligence is otherwise beyond doubt.

ARTHUR HEWSTONE,
Grey's Hospital, Pietermaritzburg.

HANOVER, 1958

The German Industries Fair opens in Hanover on the 27th April, 1958. It should prove of great interest to all who want to know the present state of technical development and production methods. Tickets cost DM. 6 (approx. 10 shillings sterling) payable in South Africa. The holder of a ticket gets a free visa into Germany.

Agents : The South African-German Chamber of Trade and Industry, Johannesburg. Tel. 227327/233722.

A. v. Falkenhausen, c/o Bellstedt & Co. (Pty.), Ltd., 9 Queen St., Durban.

INSTITUTE OF BIOLOGY

Second International Symposium on Freezing and Drying to be held in London, 1st and 3nd April, 1958. Application for programme and tickets should be made to: Institute of Biology, 41 Queen's Gate, London, S.W.7.

The Conference is organised into four sessions, each session under the chairmanship of one of the following:—

Dr. A. S. Parkes. Dr. E. W. Flosdorf. Dr. S. T. Cowan.
Prof. R. W. Rycroft.

Speakers:

Prof. B. Luyet (U.S.A.): On the Mechanism of Crystal Growth and on Vitrification and Devitrification in Aqueous Systems.

Dr. H. T. Meryman (U.S.A.): The Mechanism of Freezing in Biological Systems.

Dr. P. Mazur (U.S.A.): The Effects of Sub-zero Temperatures on Micro-organisms.

Dr. C. Polge and Dr. M. A. Soltys (U.K.): The Preservation of Trypanosomes by Freezing.

Prof. N. Kalsbukhov (U.S.S.R.): Problem of Undercooling, Freezing and Vitrifying of the Whole Organism of Animals.

Dr. Y. Obayashi (Japan): The Preservation of B.C.G.

Dr. P. W. Muggleton (U.K.): Freeze Drying of Bacteria with Special Reference to B.C.G.

Dr. G. R. Scott and Mr. C. S. Rampton (Kenya): Dried Virus Vaccines: The Problem of the Batch Size.

Dr. R. I. N. Greaves (U.K.): Some Factors which Influence the Stability of Freeze Dried Cultures.

Prof. Tokio Nei (Japan): The Effect of Freezing, Thawing and Freeze Drying upon Micro-organisms.

Mr. T. W. G. Rowe and Mrs. E. Robson (U.K.): The Physics of Secondary Freeze Drying.

Dr. J. L. Stephenson (U.S.A.): Fundamental Physical Problems in the Freezing and Drying of Biological Materials.

Prof. L. R. Rey (France): Study of the Freezing and Drying of Tissues at Very Low Temperatures.

Dr. A. S. Parkes (U.K.): Freeze-Drying of Viable Grafts: Problems and Possibilities.

Prof. D. Grieff (U.S.A.): The Effects of Freezing, Low Temperature Storage and Drying by Vacuum Sublimation on the Activities of Viruses and Cellular Particulates.

Dr. B. L. Brady (U.K.): Some Observations on the Freeze-Drying of Yeasts.

Commander G. W. Hyatt, M.D. (U.S.A.): The Storage of Human Tissues for Surgical Application.

Prof. C. G. Rob (U.K.): Experience with the Clinical Use of Freeze-Dried Arteries.

Dr. H. A. Sissons, et al (U.K.): Freeze-Dried Bone as a Grafting Material.

CORRECTION

It is regretted that the undermentioned were omitted from Vol. 3, No. 5 :—

Page 14 : After R. J. Lavington, General Secretary,
Add : Institute of Medical Laboratory Technology.

Page 15 : Before "Official Appointments"
Add : The following notices are reprinted from various sources to underline the salary scales offered elsewhere in Africa.

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